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Distribution of Bacterial Infections in the Lubbock Infection Surveillance Study of Wastewater Spray Irrigation

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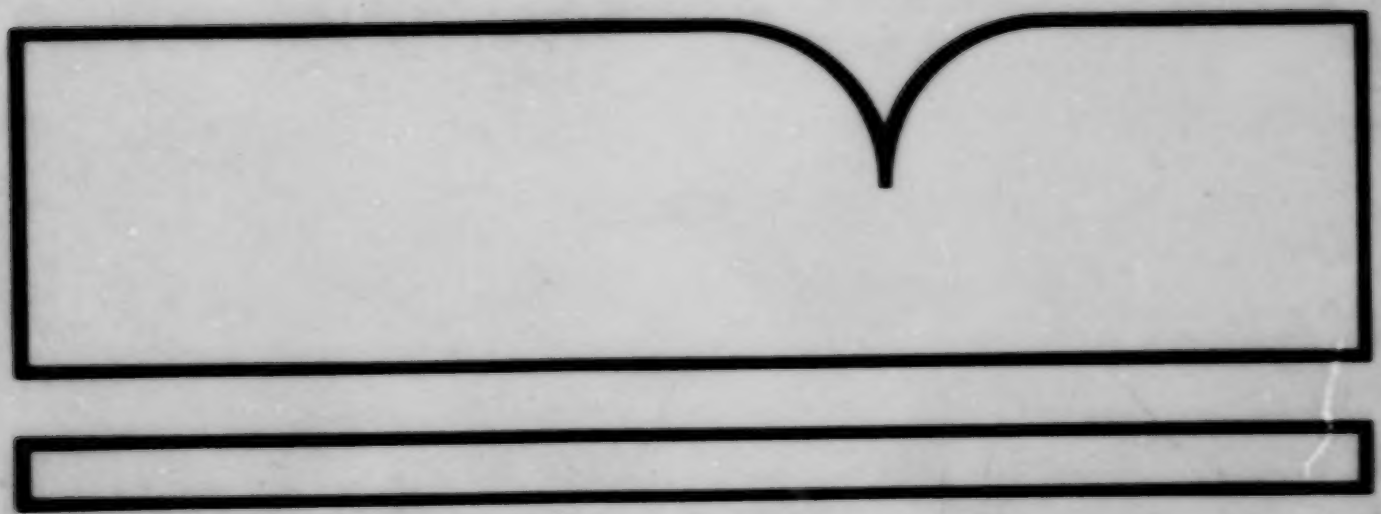

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THE DISTRIBUTION OF BACTERIAL INFECTIONS IN THE LUBBOCK INFECTION SURVEILLANCE STUDY OF WASTEWATER SPRAY IRRIGATION

by

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16. ABSTRACT

The Lubbock Infection Surveillance Study monitored bacterial infections in the vicinity of the Lubbock Land Treatment System expansion near Wilson, Texas to investigate the association, if any, between the occurrence of new bacterial infection in people and their exposure through slow rate (sprinkler) land application to wastewater or its aerosol which contained potentially pathogenic bacteria.

Bacterial infections were monitored during the first four major periods of wastewater irrigation: February-April 1982, July-September 1982, February-April 1983 and July-August 1983. Fecal specimens were provided by about 120 donors in 1982 and 100 donors in 1983 during designated weeks just before, during and near the end of each irrigation period to detect new bacterial infections occurring in the interim. Clinical bacteriological analyses were designed to isolate overt and opportunistic pathogens.

Infection by overt enteric bacterial pathogens was rarely observed. No isolates of Shigella, Campylobacter fetus sp. jejuni, or Yersinia enterocolitica were obtained. Salmonella infection was found in only one household, with no apparent relationship to wastewater irrigation.

The prevalence of Klebsiella pneumoniae infections was very high (5-10%) during both summers, but lower (1-4%) in the winter and spring of both years. Infections by a variety of other opportunistic bacteria were also detected, but much less frequently.

Aeromonas hydrophila and fluorescent Pseudomonas were prominent in sprayed wastewater, but rare in baseline fecal specimens. Prevalence of these organisms in the study population ranged from 1 to 9% during irrigation periods, with fluorescent Pseudomonas infections being more common.

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THE DISTRIBUTION OF BACTERIAL INFECTIONS IN THE LUBBOCK INFECTION SURVEILLANCE STUDY OF WASTEWATER SPRAY IRRIGATION

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INTRODUCTION

Increasing interest in water reuse as a solution to the problems of sewage wastewater disposal and water shortages has prompted a number of studies [1-5] investigating possible health hazards from land application of wastewater. A variety of agents of human disease, including many overt and potentially pathogenic microorganisms, may survive treatment processes [6,7], and thus could theoretically pose a threat. The routes of transmission include direct contact, inspiration of contaminated aerosols, contamination of food crops or animals, and contamination of surface or ground waters.

The study of Katzenelson et al. [1] cautioned that the infectious disease hazards associated with irrigation of partially treated wastewater are greater than previously assumed. Existing illness records were analyzed in a retrospective study of enteric diseases among communal agricultural settlements (kibbutzim) in Israel. The incidence rates of enteric illness for kubbutzim utilizing wastewater for spray irrigation were compared with other kibbutzim practicing no form of wastewater irrigation. Two- to four-fold increases in the incidence of shigellosis, salmonellosis, infectious hepatitis, and typhoid fever were reported for the kibbutzim utilizing wastewater, whereas the incidence of other diseases not normally associated with sewage were similar in both groups. A subsequent retrospective study of Israeli kibbutzim by Shuval et al. [4] identified serious deficiencies in the data of the original study, including misclassification of some kibbutzim regarding wastewater reuse, uncertainties about periods of irrigation, and the inadequacy of the communicable disease reports used as the basis for the study. Indeed, the subsequent study failed to find evidence of excess risk associated with wastewater irrigation except in kibbutzim in a "switch" category (i.e., in kibbutzim practicing two consecutive years of wastewater irrigation followed by the same period without irrigation or vice versa). In this category, a significantly increased risk of total enteric disease was noted only for the 0-4 age group during periods of wastewater irrigation.

Studies [5,8-12] in the United States of occupationally exposed workers and of populations living in the vicinity of activated sludge sewage treatment plants have not indicated a significant health risk associated with this type of wastewater exposure. For example, Northrop et al. [11] did not find significant correlations

between exposure indices to aerosols from an activated sludge plant and the rate of self-reported illnesses or bacterial or viral infection rates in the population living around the plant. A study by Linnemann et al. [5] of Muskegon County, Michigan workers exposed to wastewater spray irrigation failed to show any differences in illness or viral isolation rates between the workers and a control group. Although antibody titers to coxsackievirus B5 were significantly higher in spray irrigation nozzle cleaners, seroconversions were not documented. Likewise, a prospective sero-epidemiologic study by Clark et al. [12] of municipal sewer and sewage treatment workers and controls in three American metropolitan areas failed to support a significant risk associated with exposure to the wastewater, although some findings suggested a slight increase in bacterial and viral infections may occur in the sewage treatment workers.

The Lubbock Infection Surveillance Study (LISS) [13] has monitored viral and bacterial infections in a rural American community surrounding a major new land treatment demonstration project [14] utilizing spray irrigation of wastewater. The prime objective for the bacteriological investigation was to examine the association, if any, between the occurrence of new bacterial infections in participants and their exposure through slow rate (sprinkler) land application to wastewater or its aerosol which contained potentially pathogenic bacteria. This paper presents initial results and findings from extensive clinical monitoring of bacterial infection status based on monthly routine fecal specimens.

METHODS

Study Site

A major expansion [14] of the Lubbock, Texas land treatment system was constructed at the Hancock farm north of Wilson, Texas (see Figure 1). A new 25-km pipeline transported an average daily flow of 14,000 m^3 of trickling filter effluent from the Lubbock sewage treatment plant (SeWRP) to the 1500 ha Hancock farm irrigation site. The treated wastewater was applied both directly from the pipeline and from storage reservoirs, primarily by 22 center-pivot sprinkler irrigation rigs. Since operations began in February 1982, wastewater irrigation has been practiced primarily in the early spring (February 15-April 30) and in the summer (July 1-September 15).

A LISS population [13] of 439 residing in 156 households was recruited in 1980, prior to facility construction. The study households were located within a rectangular area which extended up to 4.8 km beyond the Hancock site boundary and included the town of Wilson (population 576, 1980 census). Nearly 10% of the study population had frequent wastewater contact and heavy aerosol exposure or lived within 200 m of the spray irrigation.

Study Design

The LISS conducted a health watch [13] to monitor infections and illnesses occurring in the study population from June 1980 through mid-October 1983, as illustrated in Figure 2. The study focused on infections and the infecting agent rather than on illness to obtain greater objectivity, sensitivity, specificity, and etiologic evidence.

An adult from each household and any children under age 13 were requested to submit a series of routine stool specimens whether well or ill during designated weeks just before, during and near the end of each irrigation period (see Figure 2). The bacteriological investigation was based on a semiquantitative assay of the sequential fecal specimens from a donor to detect new enteric bacterial infections by overt and oportunistic pathogens occurring between successive specimens. The possible association of the bacterial infections in appropriate categories with concurrent wastewater irrigation and alternative sources of infection was then investigated. Stool specimen donation was limited to two donors per household in 1983. Routine fecal specimens were obtained almost exclusively from children in 1980 and 1981 as a baseline.

When an overt or opportunistic bacterial pathogen was isolated from a fecal specimen at or exceeding a specified semiquantitative level which might be associated with enteric disease, the donor was considered to be infected. These levels were:

Category 1—any isolate of a major enteric bacterial pathogen (i.e., Salmonella or Shigella species, Campylobacter jejuni, or Yersinia enterocolitica);

Category 2--isolation at the heavy level of a possibly significant opportunistic pathogen (i.e., API Group I, Candida albicans, Chromobacterium, Citrobacter, Klebsiella, Morganella, Proteus, Providencia, Serratia, and Staphylococcus aureus);

Category 3--isolation at the moderate or heavy level of selected organisms found to be uncommon in feces but prominent in the sprayed wastewater (i.e., Aeromonas hydrophila and the fluorescent Pseudomonas group: P. aeruginosa, P. fluorescens, and P. putida).

The infected donor was considered to have had a new infection since donation of the prior fecal specimen, when the level of the organisms in the prior specimen had been:

1) negative, for major enteric pathogens;

2) negative to light, for possibly significant opportunistic pathogens;

3) negative to light, for organisms prominent in the wastewater.

The densities of these overt and opportunistic pathogens and indicator bacteria in the sprayed pipeline and reservoir wastewater were monitored regularly (see Figure 2). Representative data in Table 1 illustrate that the overt and opportunistic pathogens except Shigella were present periodically. Aeromonas hydrophila, the fluorescent Pseudomonas group, and Klebsiella consistently were prominent organisms in the wastewater. Pipeline wastewater always had much higher microorganism levels than reservoir wastewater.

Also, each household was contacted weekly for a report on any illnesses during the prior week. When a recent respiratory or gastrointestinal illness was reported, the ill participant was requested to submit a throat or stool specimen for clinical

bacteriological analysis. While supplementing the bacterial investigation, these data are not presented in this paper because their analysis is incomplete.

Fecal Specimen Collection

During scheduled 1-week collection periods, freshly passed stool specimens were collected by designated donors in Sage commode system containers, refrigerated, and delivered that same day to the processing center. Specimens from babies were collected from freshly soiled diapers and transferred to specimen containers. Specimens were processed by transferring approximately 10 g each of a representative portion of the specimen to two appropriately labeled specimen containers. Thirty milliliters of buffered glycerol saline transport medium [15] was added to one container and the contents were thoroughly mixed to preserve bacterial viability. Specimens were cooled to 4°C prior to packaging with ice packs in a Bio-mailer (Polyfoam Packers Corporation) in a corrugated box. The specimens were shipped by overnight express bus and arrived at the laboratory between 16 and 36 hours after collection.

Microbiological Analyses

Analyses for selected organisms in the fecal specimens were performed as summarized in Figure 3. The prevalence of different microbial types in the specimens was determined in a semiquantitative manner. All primary plating media were streaked by the same four quadrant method, and the amount of growth of each microorganism isolated was reported by determining the highest quadrant in which the organism was isolated as discrete colonies. The terminology used and respective definitions were:

Very light - 1 to 10 colonies on the plate
Light - growth in first quadrant
Moderate - growth on first two quadrants
Heavy - growth on three or all quadrants

Specimens which failed to yield any growth, or which yielded organisms by enrichment only, were excluded from the data set. The lack of organisms, in these cases, is likely to have been due to problems with sample processing, shipping or use of antibiotics by participants.

Fecal specimens in the transport medium were used for all isolations, with the exception of that for *Campylobacter jejuni* (Figure 3D) where the specimen cup containing the representative portion of the original sample was used. Contrary to some reports [15], *C. jejuni* may survive poorly in buffered glycerol saline [16], a widely used transport medium for most enteric bacterial pathogens. All media were formulated from the appropriate Difco (Detroit, Michigan) dehydrated product, with the exceptions of the cellobiose arginine lysine (CAL) agar of Dudley and Shotts [17] which was obtained from Scott Laboratories (Fiskeville, Rhode Island) and plates of Campy-BAP agar which were purchased from the same source or from BBL Microbiology Systems (Cockeysville, Maryland).

The procedure for primary isolation and identification of *Salmonella*, *Shigella*, *Yersinia enterocolitica*, and other enterics is shown in Figure 3A. CAL agar, a special purpose differential medium

for isolation of Y. enterocolitica [17], was incubated at room temperature for 48 hours. The other three media were chosen to represent three levels of selectivity for the various Enterobacteriaceae and other enterics. These were a differential medium with little selectivity (MacConkey), a differential, moderately selective medium (Hektoen enteric), and a highly selective medium (bismuth sulfite) used primarily in the search for Salmonella [15]. All plates were incubated at 35°C and examined at 24 and 48 hours. Plates were inspected, using a stereomicroscope with oblique transmitted illumination, and a representative of every colony type observed was subcultured for a purity check, oxidase testing by Kovac's method [15], and identification by the API-20E® biochemical screen (Analytab Products). To increase the chance of isolating Shigella (Figure 3B), 1 mL of each fecal specimen was transferred to 9 mL of GN broth, incubated at 35°C for approximately 18 hours, streaked to xylose-lysine-deoxycholate (XLD) agar, incubated at 35°C for 24 hours, and identified as described previously. The combination of enrichment in GN with isolation on XLD has been described as excellent for recovery of Shigella [18].

Enrichment of Y. enterocolitica (Figure 3C) was carried out by inoculating 1 mL of the fecal specimen to 9 mL of phosphate buffered saline followed by incubation at 4°C for 1 week. At days 3 and 7, 10 µL of the enrichment was mixed in 0.1 mL of 0.5% KOH in 0.5% NaCl, and then streaked to CAL plates. Representative colonies were picked and identified, as described previously, after incubation at room temperature for 48 hours.

The procedure for C. jejuni involved streaking Campy-BAP plates, which then were incubated at 42°C for 48 hours in a GasPak® container with the CampyPak II® (BBL Microbiology Systems, Cockeysville, Maryland) atmosphere generator. The organism was presumptively identified by the following criteria: Gram-negative curved rods, characteristic darting motility, oxidase +, and catalase +. The organisms were confirmed by growth in 1% glycine, lack of growth at 25°C, and susceptibility to nalidixic acid (30 µg disk).

The fungal yeast pathogen Candida albicans was isolated (Figure 3E) by streaking to plates of Sabouraud dextrose agar supplemented with 50 µg/mL of chloramphenicol (Calbiochem), followed by incubation at 35°C for 48 hours. Plates were inspected for white, convex, opaque colonies which were confirmed as C. albicans by germ-tube formation in bovine serum, chlamydospore production on cornmeal Tween 80® agar, and sucrose assimilation on agar slants of Wickerham's yeast nitrogen base supplemented with the sugar.

Staphylococcus aureus was isolated by streaking to plates of mannitol salt agar. Mannitol-positive colonies were picked for confirmation by examination of Gram-stained smears for characteristic morphological groups of Gram-positive cocci and positive coagulase reaction.

Wastewater Exposure Measures

The aerosol levels of bacterial indicators produced by wastewater spray irrigation were higher in 1982 by one to two orders of magnitude than in 1983 because most of the wastewater in 1982 was applied directly from the pipeline. Thus, exposure of the study

population to wastewater bacteria presumably was considerably higher during both irrigation seasons in 1982 than during either irrigation season in 1983.

Relative measures of cumulative exposure to the wastewater and wastewater aerosol were calculated for each participant within each irrigation season from data obtained by aerosol sampling, aerosol dispersion modeling, activity diaries, and a weekly log of extensive contacts. Each participant was placed in the high, intermediate, or low aerosol exposure group within each irrigation season based on his computed aerosol exposure index during that season

Other Sources of Infection

Eating habits and drinking water also were investigated as possible alternative sources of observed bacterial infections, besides wastewater irrigation. The frequency with which each fecal donor ate food prepared at each of the four eating establishments in Wilson during each irrigation period was determined through a retrospective survey.

Almost every rural household received its drinking water from a nearby well. Tap water samples were obtained approximately bimonthly from a subset of households with fecal donors (eight households in 1982 and sixteen households in 1983) and analyzed for indicator bacteria. A well was classified as contaminated for the purpose of this analysis if the sample average per 100 mL exceeded 20 for total coliforms, 2 for fecal coliforms, or 5 for fecal streptococci or if Salmonella was present in any sample. Approximately half the sampled wells were contaminated each year based on these criteria.

Epidemiologic Analysis of Associations

A descriptive epidemiologic analysis of the infections by each agent group was performed according to the person, place and time model. The prevalence of infections was calculated by specimen collection month.

An exploratory analysis was conducted to identify possible risk factors for each agent group. During the entire time interval when infections were being observed, the infected donors were compared to the donors who were not infected with regard to demographic, socioeconomic, lifestyle, drinking water and health history characteristics. When a difference was observed, the characteristic was considered a possible risk factor.

Each episode of bacterial infection which coincided with an irrigation period was examined. Cross-tabulations were performed to investigate separately the association of bacterial infections with wastewater exposure measures, eating at local restaurants, contaminated drinking water, and the apparent risk factors. Possible associations among significant factors were also evaluated to assess imbalances which might influence or confound the association with wastewater exposure.

Associations were investigated by contingency table analysis. The null hypothesis of independence of the two classifications was evaluated by the approximate chi-square test statistic using

Cochran's cell size rule and Yates' continuity correction for 2x2 tables, and by Fisher's exact test statistic when there were few observations. All associations having nominal p-values below 0.05 are reported; less significant associations consistent with the literature are also mentioned.

The p-values from the tests for association of new infections with wastewater exposure are the only ones which can be interpreted as in a test of hypothesis. Because numerous characteristics were examined in an exploratory manner and the available cell sizes were small, all identified associations must be interpreted cautiously.

RESULTS

Infections by Overt Pathogens

No major bacterial enteric pathogens were isolated from the direct platings of the 369 routine fecal specimens collected during the baseline preirrigation periods. However, Y. enterocolitica was isolated after enrichment from three different individuals in June and July 1981. Likewise, the analysis of 1,091 routine fecal specimens collected from participants after commencement of spray irrigation failed to reveal major bacterial enteric pathogens, except for the isolation of a serologically confirmed Salmonella group C_1. The organism was isolated at the heavy level from an adult male in June 1982. Subsequent requested fecal specimens also yielded this organism from the same individual and his son. Because so few infections by overt enteric bacterial pathogens were observed from routine fecal samples during the preirrigation and irrigation periods, the data were not subjected to further analysis.

Infections by Opportunistic Pathogens

A single genus, Klebsiella, produced most of the observed infections by the possibly significant opportunistic bacterial pathogens. Since more definitive risk factors and etiology might be identified for a more specific group of organisms, the Klebsiella infections were analyzed separately from the infections by the other opportunistic pathogens.

Klebsiella. Klebsiella pneumoniae was the agent recovered in 91% of the Klebsiella infections. The others observed were due to K. oxytoca. Table 2 shows that Klebsiella infections occurred throughout 1982 and 1983, but were more prevalent during both of these summers.

In contrasting the 37 fecal donors having Klebsiella infections with the 71 donors not experiencing Klebsiella infections during the same period of observation, gender was the only factor which appeared to be significantly associated with the infected donors (see Table 3). Whereas 34% of all donors had Klebsiella infections, 44% of the female donors experienced Klebsiella infections, which is a nominally significant association at the p=0.02 level. This excess of Klebsiella infections among female donors relative to male donors occurred at all age levels. An equally high proportion (i.e., 50%) of males aged 65 and above had Klebsiella infections, but this association was only of borderline significance (p=0.07). Repeated Klebsiella infections were observed over intervals ranging

up to 20 months in 12 donors, 10 of whom were females. Hence being female appears to be a risk factor for infection by Klebsiella in the population studied.

Episodes of Klebsiella infection coincided with two of the major wastewater irrigation periods: summer 1982 and summer 1983. As Table 4 indicates, there is no evidence that the 13 new Klebsiella infections observed in summer 1982 were associated with wastewater irrigation. The percentage newly infected among donors who had a high level of wastewater aerosol exposure (6%) was lower than among the less exposed donors.

The 12 new Klebsiella infections observed during summer 1983 were associated both with wastewater aerosol exposure ($p=0.03$) and with eating frequently at restaurant A ($p=0.03$). The percentage with new Klebsiella infections was 26% among donors with high aerosol exposure, 11% with intermediate aerosol exposure, and 7% with low aerosol exposure. The Klebsiella infections among female donors appeared more likely to be associated ($p=0.01$) with aerosol exposure: 44% newly infected in the high group, 17% intermediate, and 0% low. The apparent association of the Klebsiella infections during summer 1983 with wastewater aerosol exposure was stronger than with direct wastewater contact or time spent on the Hancock farm. This suggests that the wastewater aerosol was a more plausible source of exposure than the other routes investigated.

However, these Klebsiella infections occurring in summer 1983 were also associated with eating at least once per month at restaurant A. One third of the donors who ate there that frequently were infected. Furthermore, a part-time food handler at restaurant A had prolonged (or repeated) Klebsiella infections observed in August and September 1982, in February 1983, and again in July and August 1983. Thus, this food handler was experiencing a Klebsiella infection while working part-time at restaurant A when the others became infected.

Aerosol exposure was very highly associated ($p<<0.0001$) with eating at restaurant A among the fecal donors in summer 1983. The five donors with Klebsiella infections from the high aerosol exposure group were the same five infected individuals of those who ate at restaurant A more than once per month. Since there were no other infected individuals who had high aerosol exposure or ate frequently at restaurant A, the data do not permit any inference regarding whether aerosol exposure or eating at restaurant A was the actual risk factor in this Klebsiella infection episode.

Other Opportunistic Pathogens. Infections by a variety of other possible opportunistic microbial pathogens also were detected: Staphylococcus aureus (27%), Citrobacter freundii (20%), Citrobacter diversus (13%), and 7% each by API Group I, Candida albicans, Morganella morganii, Proteus mirabilis, Serratia fonticola, and Serratia liquefaciens. These infections occurred sporadically throughout the study (see Table 2). Donors who ate at restaurant B experienced significantly more of these infections (see Table 3). While not significantly associated ($p=0.11$) perhaps because of the small sample size, two (33%) rural donors drinking contaminated well water had these opportunistic bacterial and fungal infections, while none of 11 rural donors drinking well water of better quality were infected.

An episode of infections by these opportunistic microorganisms occurred in the early spring of 1983 (see Table 4). While unrelated to any measure of wastewater exposure, it did appear to be associated with eating at least once per month at restaurant B.

Infections by Bacteria Prominent in Wastewater

The donor population experienced 27 infections by Aeromonas hydrophila and the fluorescent Pseudomonas species, some of the most prevalent enteric bacteria in the sprayed wastewater. Most (89%) of these infections were by the fluorescent Pseudomonas group (P. aeruginosa, P. fluorescens, and P. putida). As Table 2 shows, these infections occurred throughout 1982 but were more prevalent in 1983, when all of the A. hydrophila infections occurred.

The characteristics associated with the donors experiencing fluorescent Pseudomonas and A. hydrophila infections are presented in Table 3. The infected donors exhibited a pattern of characteristics associated with the elderly: age 65 and above, living alone, retirees and homemakers who spent the day at home, infrequent contact with large groups of people, previous gastrointestinal conditions, and previous heart conditions. Because many of these were characteristics of the same infected donors, the data do not permit inference as to which one(s) may be actual susceptibility or exposure risk factors. Repeated or prolonged infections were observed in seven donors, six of whom were older than 60.

Drinking more water than others their age also appeared to be significantly associated with the infected donors. However, the quality of the drinking water of rural households with private wells was not associated with these infections in the subset of donors whose well water was monitored. Whereas two (20%) of the donors whose private wells were contaminated with the bacterial indicators experienced infections by these prominent wastewater bacteria, three (38%) of the donors who drank well water of better quality also had these infections.

Episodes of infection by bacteria prominent in the wastewater occurred during three of the four wastewater irrigation periods monitored. The findings from the analyses of these episodes are summarized in Table 5. There is no evidence that the minor episode in the early spring of 1982 was associated with the initial irrigation period.

While the fluorescent Pseudomonas infections in the summer 1982 episode occurred in donors with high and intermediate wastewater aerosol exposure, the pattern was not pronounced enough to be statistically significant ($p=0.26$). However, three characteristics from the "elderly pattern" did appear associated with these infected donors: living alone, a history of gastrointestinal conditions, and a history of heart conditions.

The largest episode of infection by the prominent wastewater bacteria occurred in summer 1983. It was not associated with exposure to the wastewater aerosol or any other aspect of the wastewater irrigation. However, lack of frequent contact with large groups of people appeared to be associated with this infection episode.

DISCUSSION

The study of Katzenelson et al. [1] in Israel, which showed a marked increase in the incidence of selected communicable enteric diseases in kibbutzim practicing wastewater spray irrigation compared with kibbutzim that did not, sparked concern about the health effects of the land application of wastewater. In contrast, minimal or no effect has been observed in more recent health effects studies in the United States of populations exposed to aerosols from activated sludge plants [8-11], of workers exposed to wastewater spray irrigation [5], and of municipal wastewater treatment and sewer maintenance workers [12]. In fact, a more complete retrospective study of the incidence of enteric disease associated with wastewater utilization by kibbutzim in Israel [4] raised serious questions about the results of the original study of Katzenelson et al. [1]. An excess risk of enteric disease was not associated with wastewater irrigation except in the 0-4 age group of kibbutzim of a "switch" category during periods of wastewater irrigation compared to periods during which wastewater was not used. This excess risk of total enteric disease ranged from 32 to 112% in this single group, a finding far different from the two- to four-fold increase of cases of salmonellosis, shigellosis, typhoid fever, and hepatitis reported for the kibbutzim practicing wastewater irrigation in the first study.

Jakubowski [19] has critically reviewed and evaluated the above health effects studies and has noted that the preponderance of data was negative. However, he observes that interpretation of the significance of the data, whether negative or positive, of all the studies is limited by the low numbers of highly exposed persons and the inability to adequately and quantitatively determine that exposure. None of the previous studies has investigated the health effects on populations exposed to sprinkler systems that apply wastewater to land according to EPA design criteria. The LISS was designed for this purpose and to answer many of the criticisms of previous studies, such as the reliance on self-reported illness, long-recall surveys or retrospective analysis of health data. The site was unique in that a typical rural community, without prior exposure to wastewater, was challenged by enteric agents active in a much larger urban community.

The LISS involved a variety of health watch activities (Figure 2) including serology for viruses present in the wastewater, routine fecal specimens for bacteriological and virological analyses, analyses of illness specimens, tuberculin skin testing, household health reports, and activity diaries. The health watch activities were supplemented by environmental monitoring of aerosols, wastewater, and drinking water. This study differs from previous studies in that both illness and infection were monitored, but primary emphasis was placed on the infection monitoring including extensive bacteriological analyses of fecal specimens. It was assumed that apparent disease might constitute only a small part of the total number of infections that might occur during wastewater irrigation. Thus, methods were designed to not only rigorously search for overt enteric bacterial pathogens such as Salmonella, Shigella, Yersinia enterocolitica, and Campylobacter jejuni, but also for heavy colonization by important opportunistic pathogens and for unusual occurrences of organisms which were prominent in wastewater but rare in fecal specimens from initial baseline monitoring.

It should be noted that we are not equating infection with disease, the latter being indicated by detectable alterations in normal tissue functions (i.e., clinical manifestations of illness). Infection is used in the broader sense of the entrance and multiplication of a microbe in the body. The organisms of our first category, overt enteric pathogens, are of major clinical significance because they often are associated with disease and even inapparent or subclinical infections may provide a source for infection and disease in others. In spite of a rigorous search for overt enteric bacterial pathogens, the number of isolations from the routine fecal specimens was small in baseline monitoring (three) and periods after commencing of irrigation (one). Overt pathogens often were detected in the wastewater sampling with the exception of Shigella, which may have been below the level of detection by the direct plating and enrichment procedures used. The size of inoculum required to produce disease in humans varies widely for enteric pathogens [20], ranging, for example, from as few as 10 organisms for Shigella to 10^8 for most serotypes of Salmonella.

The clinical significance of fecal isolates of the organisms at levels defining the other two categories is questionable. However, opportunistic pathogens were infrequently isolated at levels defining Category 2 during baseline fecal sampling and only 0.3% of the baseline samples yielded isolates meeting the definition of Category 3. These observations coupled with the prominence of some of the organisms (particularly Aeromonas hydrophila, the fluorescent Pseudomonas group, and Klebsiella pneumoniae) in wastewater led the authors to believe that the two categories might provide a sensitive indicator of a possible health risk associated with exposure to wastewater. In addition, the organisms may be associated with enteric disease if isolated in large numbers from stools. For example, enterotoxin producing Klebsiella, Enterobacter, Proteus, Citrobacter, Serratia, and Aeromonas have been isolated from the stools of children and infants with acute gastrointestinal symptoms [21]. Some K. pneumoniae and Enterobacter cloacae produce heat stable (ST) and heat labile (LT) enterotoxins, the latter of both organisms being immunologically related to cholera toxin and Escherichia coli LT [22]. K. pneumoniae ST recently has been purified to homogeneity and found to have the same potency as E. coli ST in the suckling mouse assay and immunological cross-reactivity with the E. coli toxin [23]. Likewise, A. hydrophila produces an enterotoxin, and the organism has been associated with diarrhea in American travelers, but not in Thais [24]. A large percentage (41%) of A. hydrophila isolates from diarrheal stools were negative for enterotoxin in a recent study [25] and enterotoxicity was approximately equally divided (i.e., 58% and 53%) among fecal and environmental isolates. It is apparent, however, that the quantitative importance of the organisms of Categories 2 and 3 in enteric disease is probably small and the etiological role of many of the organisms as enteric pathogens is not well established. Many of the organisms of Categories 2 and 3 do have unquestioned roles as major nosocomial pathogens [26].

A number of observations relating to nosocomial infections (NIs) by organisms of Categories 2 and 3 are perhaps relevant to the present study. The association of Klebsiella infections with elderly males, albiet borderline significant, and the significant association of prominent wastewater bacterial infections with the elderly (Table 3) may be related to the observation that the elderly

are at increased risk for acquiring NIs. Gross et al. [27] noted that of all NIs, 64% occurred after 60 years of age even though the elderly group represented only 23% of hospitalized patients. Increased prevalence and levels of intestinal colonization by organisms such as Klebsiella in a hospital environment have been associated with severity of illness, duration of hospitalization, and use of antibiotics. For example, Haverkorn and Michel [28] observed that the rate of intestinal colonization by Klebsiella in patients in a urological ward increased during the first 3 or 4 weeks of residence in the wards. Goldman et al. [29] observed that the chance of babies acquiring Klebsiella, Enterobacter, and Citrobacter (KEC) correlated with duration of stay in the neonatal intensive care unit (ICU). The organisms (presence/absence) were isolated from the stools of 2% of babies on admission, from 60% at 15 days, and from 91% of those remaining in the ICU for 30 days. Goldman et al. [29] also observed that broad spectrum antibiotic therapy predisposed babies to colonization of the intestine with KEC. The major effect of antibiotics on infections by such opportunistic pathogens would be to reduce susceptible components of the indigenous flora that normally play a role in prevention of colonization of other organisms through "bacterial interference." Seldon et al. [30] observed that antibiotic therapy predisposed patients to intestinal colonization (presence/absence) with Klebsiella and exerted a selective pressure in favor of multidrug resistant Klebsiella. They observed that 25% of patients on acute care wards were intestinal carriers of multidrug resistant Klebsiella compared to only 1.9% of 54 physicians and nursing personnel.

At least five possible causes of the elevated levels of Klebsiella and other opportunistic pathogens and the unusual isolations of organisms in Category 3 are suggested by the observations from NIs and other reports. These include:

1) antibiotic selection of resistant organisms or promotion of growth as a result of reduction of competing flora by prior use of antibiotics,

2) ingestion of organisms on garden vegetables,

3) exposure to Gram-negative bacteria associated with heavily contaminated cotton,

4) fecal contamination of drinking water,

5) wastewater irrigation operations.

Antibiotic selection or promotion of growth is an unlikely cause of the isolations in Categories 2 and 3 since the isolations were observed with routine rather than illness specimens. Ingestion of contaminated garden vegetables also is an unlikely cause, even though Wright et al. [31] reported that salads may be heavily contaminated with Gram-negative bacilli. Wright et al. [31] studied the flora of foods served to patients in a hospital and recovered enteric bacteria and Pseudomonas aeruginosa from vegetable salads. The organism most frequently isolated was Enterobacter agglomerans (85% of samples, 10^2-10^6 CFU/g). Other organisms isolated frequently and mostly at high counts were E. cloacae (48%) and Klebsiella (46%). The studies of Casewell and Phillips [32] and Cooke et al. [33] challenge some of the interpretations of Wright et al. [31]. Casewell and Phillips [32] observed that food prepared

for intensive care patients was frequently contaminated with Klebsiella but noted that the hospital was the main source of contamination. Likewise, Cooke et al. [33] examined hospital food for the presence of Klebsiella. Salads and cold meat were the most frequently contaminated foods. However, Klebsiella also was widely distributed in the hospital kitchen environment which was considered, at least in part, to be the source of the organisms found in the food. It should be noted that E. agglomerans, the most frequent isolate from salads in the study of Wright et al. [31], was isolated at any level of growth from less than 1% of the fecal specimens of LISS participants.

Exposure to Gram-negative bacteria associated with heavily contaminated cotton also is an unlikely cause of the unusual isolations. Morey et al. [34] recently reported that seed cotton and cotton plants collected from Lubbock, Texas were heavily contaminated with Gram-negative bacteria. The organisms were not identified; however, the investigators noted that E. agglomerans was the predominant species in other similar studies. E. agglomerans is a relatively recent designation for a group of organisms which include the former Herbicola-Lathyri bacteria which were included in the plant associated genus Erwinia. The nature of the flora of the contaminated cotton and the lack of relationship to the isolations from specimens of LISS participants make contaminated cotton an unlikely source. It should also be noted that while K. pneumoniae is widely distributed in the environment, strains isolated from humans and animals may be routinely different in properties. Bagley and Seidler [35] noted that 85% (49/58) of K. pneumoniae of human and bovine origin were fecal coliform (FC) positive whereas 16% (19/120) of environmental strains were FC positive. Strains of K. pneumoniae that are FC positive have been shown to have other unique properties [36].

The fact that the unusual isolations of organisms of Categories 2 and 3 occurred over a defined period also tends to argue against possibilities 1 through 3, but not 4 and 5. Fecal contamination of drinking water as a consequence of contaminated individual wells and city of Wilson water is a possibility since most of the isolations occurred following a period of unusually heavy (>10 in.) rainfall in the Lubbock area in May and June 1982. Klebsiella has been reported to be the most prevalent, potentially pathogenic Gram-negative bacterium in the air surrounding sewage treatment plants [37,38] and in air samples of wastewater used for spray irrigation [5]. The organism also is found at very high levels in textile finishing plant effluents [39] and in pulp and paper mill effluent discharge [40], and thus may be expected in the aerosols of those sources as well.

Much of the interest in aerosols associated with sewage treatment and land application of wastewater has centered around small particle aerosols (i.e., 5 um or less) which may be carried to deep areas of the lungs. However, it has been proposed that most human bacterial pneumonia is due to microorganisms that have colonized the oropharynx, and that aspiration of such organisms may be the principal mechanism underlying nosocomial pneumonia [41]. An interesting observation in experimental animals (mice and monkeys) was that K. pneumoniae administered by aerosol was significantly less virulent than when given by intranasal or intratracheal instillation [42]. These observations suggest that large particle

aerosols containing the organism may lead to colonization of the nasopharynx associated with seeding of the gut by the organisms.

An apparent association of Klebsiella infections with wastewater aerosol exposure occurred in summer 1983 (Table 4). However, eating frequently at restaurant A was equally associated with this infection episode and in the same individuals. The restaurant etiology may be more compelling for two reasons. Firstly, a part-time food handler at restaurant A was infected by Klebsiella during the same period, and secondly, the Klebsiella infections in summer 1982 were not associated with aerosol exposure, even though wastewater aerosol levels of Klebsiella were higher in summer 1982. However, the summer 1982 association could have been obscured by heavy rainfall-associated contamination of drinking water which occurred in that period.

The results reported in this paper, dealing with the incidence of infection inferred by isolation of either overt or opportunistic pathogens from fecal specimens, do not appear to suggest an increased risk associated with exposure to wastewater. However, the results are preliminary and should be considered in light of the additional parameters included in the health watch of the LISS and a more rigorous statistical analysis of data presented in this paper.

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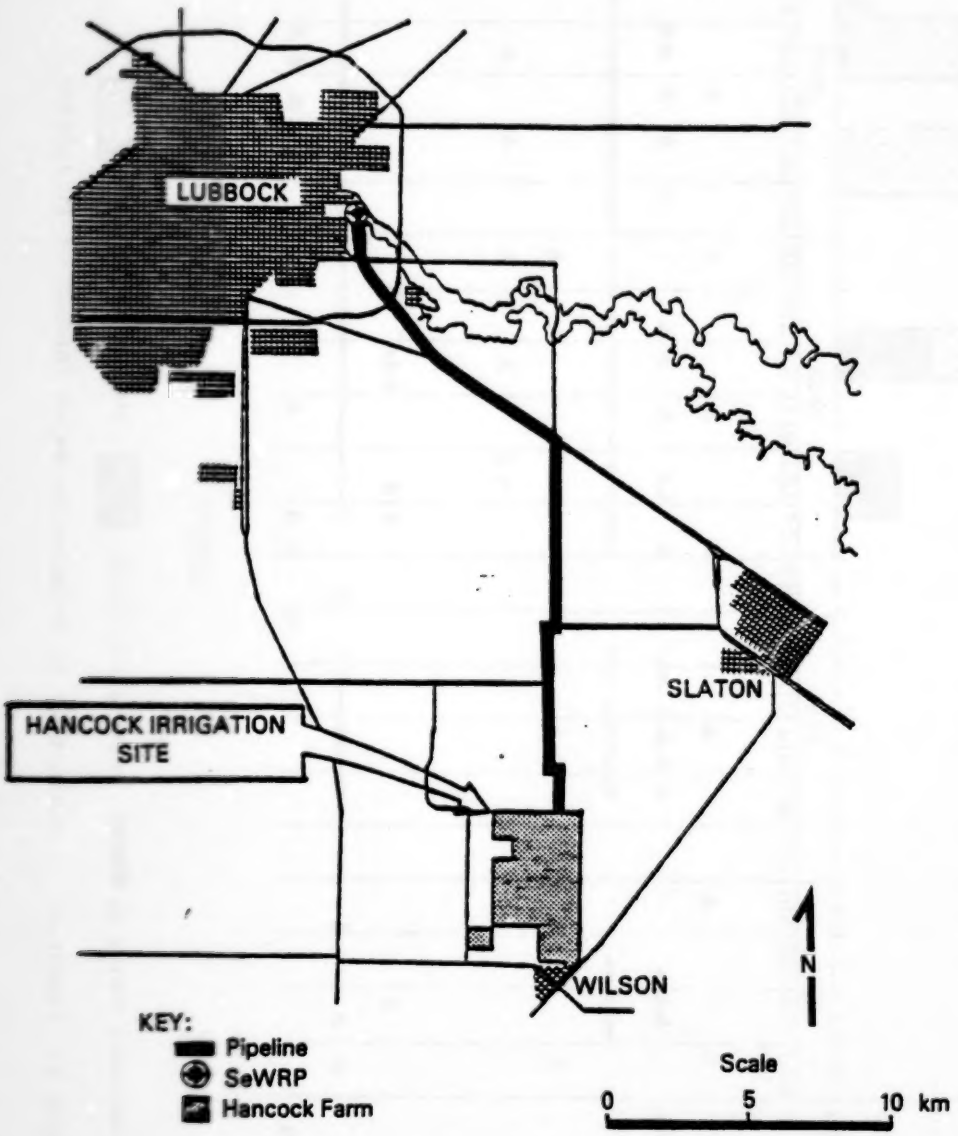


Figure 1. Location of Wastewater Source and Irrigation Site

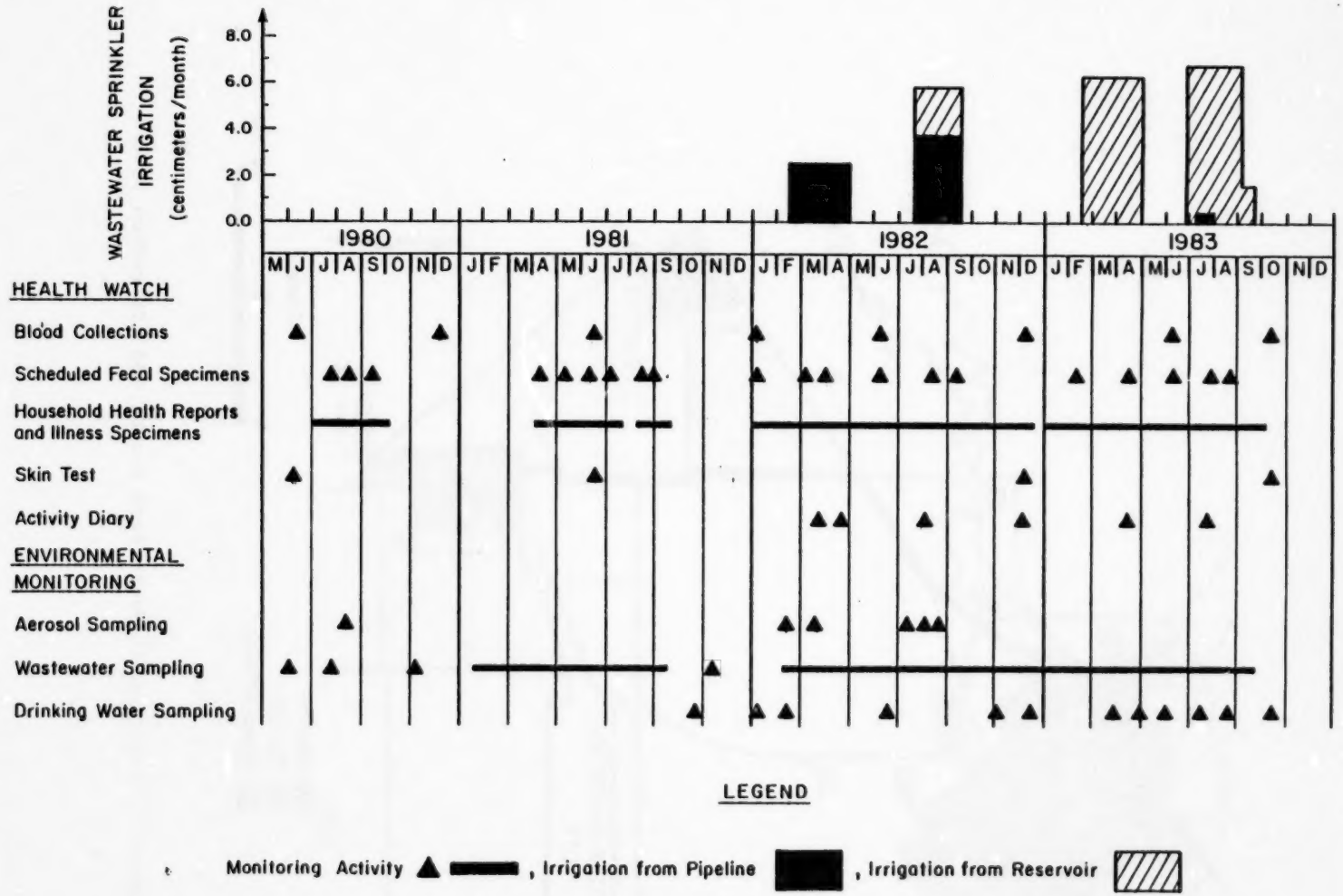
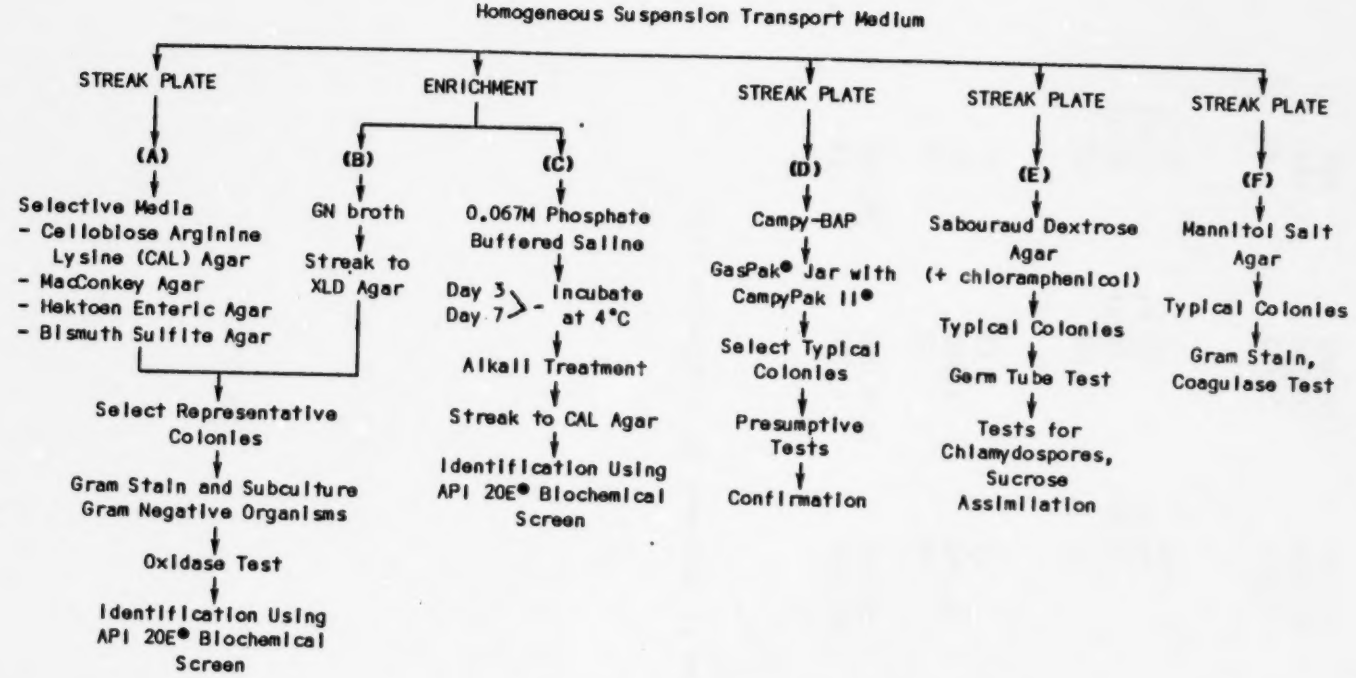


Figure 2. Timeframe of Monitoring in Relation to Major Periods of Irrigation



(A) Salmonella, Shigella, Yersinia enterocolitica, other enterics
(B) enrichment for Shigella
(C) enrichment for Y. enterocolitica
(D) Campylobacter jejuni
(E) Candida albicans
(F) Staphylococcus aureus

Figure 3. Isolation and Identification of Selected Organisms from Feces

TABLE 1. REPRESENTATIVE BACTERIAL CONCENTRATIONS (cfu/mL) IN WASTEWATER[a]

	Pipeline Effluent		Reservoir
	Mar 22, 1982	Jul 26, 1982	Jul 26, 1982
Bacterial Indicators			
Total coliforms	57,000	120,000	500
Fecal coliforms	81,000	58,000	190
Fecal streptococci	7,900	2,300	3
Overt Pathogens			
Salmonella sp.	100	≥0.1	<0.01
Shigella sp.	<0.01	<0.01	<0.01
Yersinia enterocolitica	<0.01	<0.01	<0.01
Campylobacter jejuni	<3	10	<3
Opportunistic Pathogens			
Candida albicans	<3	<3	<3
Klebsiella sp.	50,000	5,000	30
Staphylococcus aureus	<3	<3	<3
Aeromonas hydrophila	≥8,000	≥150,000	≥4,300
Fluorescent Pseudomonas	260,000	6,000	13

a 24-hour composite samples of influent to irrigation rigs.

TABLE 2. PREVALENCE OF BACTERIAL INFECTIONS BY COLLECTION MONTH

Specimen Collection Month	Routine Fecal Donors	Prevalance Rate (Infections per 100 donors) Opportunistic Pathogens Klebsiella	Opportunistic Pathogens Others	Bacteria Prominent in Wastewater
1980				
Jul	22	0	0	0
Aug	36	5.6	0	0
Sep	47	0	0	0
1981				
Apr/May	27	0	3.7	0
Jun	44	2.3	0	0
Jul	29	0	0	0
Aug/Sep	35	0	5.7	0
1982				
Jan	105	1.0	0	1.0
Mar	125	1.6	0	0.8
Mar/Apr	118	0.8	0	1.7
Jun	124	8.1	0	0.8
Aug	107	10.3	1.9	1.9
Sep	110	8.2	0	2.7
1983				
Feb	97	4.1	1.0	5.2
Apr	107	1.9	4.7	3.7
Jun	100	3.0	0	0
Jul	103	4.9	1.9	8.7
Aug	101	9.9	2.0	3.0

TABLE 3. EXPLORATORY ANALYSIS OF THE ASSOCIATION OF INDIVIDUAL CHARACTERISTICS WITH INFECTION PREVALENCE

	Klebsiella	Other Opportunistic Pathogens	Bacteria Prominent in Wastewater
Period of observation	Jan-Sep 1982 Feb-Aug 1983	Apr-Sep 1981 Aug-Sep 1982 Feb-Aug 1983	Jan-Sep 1982 Feb-Aug 1983
Donors infected	37 (34%)	14 (16%)	19 (18%)
Donors not infected[a]	71	72	85
Characteristics associated with infected donors:			
Associated subgroup % infected (p-value)	Female 44% (0.02) Elderly male 50% (0.07)	Ate at restaurant B 31% (0.007)	Elderly ($\geq$65)[b] 38% (0.02) Drinks much water 47% (0.004) Lives alone[b] 50% (0.001) At home during day[b] 39% (0.04) Seldom in large groups[b] 32% (0.007) Gastrointestinal condition history[b] 43% (0.003) Heart condition history[b] 36% (0.01)

a No infection detected during period of observation; fecal specimens were observed in at least half (i.e., six) of the specimen collection periods.

b Confounding among age, household size, occupation, group contact, heart conditions, and gastrointestinal conditions; only one of these factors may actually be related to donors infected with bacteria prominent in wastewater.

TABLE 4. ASSOCIATION OF EPISODES OF INFECTION BY OPPORTUNISTIC BACTERIAL PATHOGENS WITH WASTEWATER AEROSOL EXPOSURE AND OTHER FACTORS

	Klebsiella Infection Episodes		Other Opportunistic Bacteria Episode
Irrigation Period	Summer 1982	Summer 1983	Spring 1983
Infection status during irrigation period			
Newly infected[a]	13(5)[b](15%)	12(8)[b](13%)	5(5)[b](5%)
Not infected[c]	75	81	102
Association with wastewater aerosol exposure (p-value)	No	Yes[d] (0.03)	No
Percentage newly infected, by aerosol exposure group			
High	6%	26%[d]	5%
Intermediate	19%	11%	5%
Low	14%	7%	4%
Characteristics associated with newly infected donors			
Associated subgroup % infected (p-value)[e]	(None)	Females with high aerosol exposure[d] 44% (0.01) Ate frequently at restaurant A[d] 33% (0.03)	Ate frequently at restaurant B 27% (0.009)

a Infected during irrigation period, but not infected in previous month.

b Onset of new infections in parentheses was definitely after irrigation commenced.

c Neither specimen during irrigation period contained the pathogen at a level classified as infected.

d Strong confounding of aerosol exposure with eating at restaurant A ($p \ll 0.0001$). Females experienced most of the Klebsiella infections among those highly exposed to both factors.

e Simultaneous influence of other possible factors is not taken into account.

TABLE 5. ASSOCIATION OF EPISODES OF INFECTION BY BACTERIA PROMINENT IN WASTEWATER WITH WASTEWATER AEROSOL EXPOSURE AND OTHER FACTORS

Irrigation Period	Infection Episodes by Bacteria Prominent in Wastewater		
	Spring 1982	Summer 1982	Summer 1983
Infection status during irrigation period			
Newly infected[a]	3(2)[b](3%)	4(3)[b](4%)	9(0)[b](10%)
Not infected[c]	110	85	85
Association with wastewater aerosol exposure (p-value)	No	No (0.26)	No
Percentage newly infected, by aerosol exposure group			
High	6%	11%	11%
Intermediate	0%	4%	9%
Low	6%	0%	11%
Characteristics associated with newly infected donors			
Associated subgroup % infected (p-value)[d]	(None)	Lives alone 18% (0.03)	Seldom in large groups 21% (0.003)
		Gastrointestinal condition history 17% (0.006)	
		Heart condition history 13% (0.02)	

a Infected during irrigation period, but not infected in previous month.

b Onset of new infections in parentheses was definitely after irrigation commenced.

c Neither specimen during irrigation period contained the pathogen at a level classified as infected.

d Simultaneous influence of other possible factors is not taken into account.